

SUMMATION AND INHIBITION FOLLOWING CONTRALATERAL STIMULATION OF THE TARSAI CHEMORECEPTORS OF THE BLOWFLY

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In one of the efforts to arrive by behavioral methods at an understanding of the fundamental process of stimulation of tarsal chemoreceptors by unacceptable compounds it was discovered that the action of opposing stimuli, as for example sugar and alcohol, could be either ipsilateral or contralateral (Dethier, 1950). Normal proboscis extension in response to sugar could be prevented by adding propanol to sucrose solutions or by stimulating one leg with propanol alone while the opposite leg was exposed to sucrose. The fact that prevention of proboscis response could result from interaction in the central nervous system of opposing patterns of activity from opposite legs directed attention to the existence of central inhibition in this insect.

In addition to providing information relative to the interaction of *unlike* stimuli, the early experiments yielded data which pointed to the existence of central summation following stimulation by *like* stimuli. However, another series of experiments in which contralateral stimulation by like stimuli was investigated for the olfactory sense (Dethier, 1952a) suggested that the observed results might be simply an expression of a statistical bias introduced by doubling the available number of receptors. When rejection thresholds for pentanol vapor were compared in flies with a single antenna and those with both antennae, it was observed that thresholds were lower for bilateral stimulation than for unilateral; but the decrease of bilateral over unilateral was never greater than could be satisfactorily accounted for on a simple probability basis (*cf.* Smith and Licklider, 1949). Thus, while these tests did not negate the existence of true neural contralateral summation, they did not necessarily affirm it. Since the experiments were designated to measure the per cent response at only one or two test concentrations rather than to ascertain values for complete threshold frequency distribution, a rigorous statistical analysis was not possible. Nor did it appear feasible, because of the difficulty of controlling vapor concentrations, to attempt to resolve the problem with the antennal preparation. Tarsal chemoreceptors are clearly more amenable to complete analysis. Consequently, the investigation described herein was undertaken to extend earlier tarsal as well as olfactory data in an effort to permit a clearer statement of the status of contralateral summation and inhibition in the response of the blowfly to chemicals.

METHODS

The solution of two immediate problems was undertaken: (1) to determine whether or not simultaneous stimulation of opposite legs by like stimuli sums in the central nervous system; (2) to determine to what extent unlike stimuli inhibit each

other. Experimental methods fell into two categories. In the first, tests were conducted with one-legged and two-legged flies. These insects were prepared as follows. One- to two-day old flies (*Phormia regina*) from a stock culture were anesthetized with carbon dioxide and attached by the wings to wax-tipped applicator sticks. In the case of one half of the flies, all legs except one prothoracic were fastened into the wax. Of this group of flies, one half had the right prothoracic leg free and the other half, the left. In the remaining group of flies all legs except the prothoracic pair were immobilized. Measurements were then made of the responses of one-legged and two-legged flies to sucrose, propanol in water, propanol in 0.1 M sucrose, and HCl in water.

In the second series of experiments the responses of two-legged flies were tested in partitioned dishes. The vessels used were Stender dishes 45 mm. in diameter with a cover glass 40 mm. long fastened on edge across the middle with paraffin. The top of the cover glass was set flush with the top edge of the dish. A different solution could now be poured into each half of the dish, care being taken to insure that the liquids rose only to such a level as to make concave rather than convex menisci at the partition.

With this device it was possible to stimulate opposite prothoracic legs of a fly simultaneously with different solutions by causing him to straddle the partition. In practice, execution of a test required the utmost delicacy and the patience of Job. A captive fly suspended in air had a tendency to keep its legs crossed or flexed at the tibio-tarsal joint. A proper test cannot be made until both legs are extended and spread apart. When a fly had assumed this position, it was lowered gently over the partition. Care had to be exercised that both legs touched the respective solutions at approximately the same moment and that the partition was not grasped by either leg. Under favorable conditions, proboscis extension, if adequate stimulation was supplied, occurred in the usual manner.

This technique was employed to test unilateral versus bilateral stimulation by a single compound and to investigate the effects of opposing stimuli acting simultaneously on opposite legs. In the first instance, unilateral stimulation, by sucrose, for example, was accomplished by forcing the fly to straddle a partition which separated a sucrose solution from pure water. For bilateral stimulation sucrose was placed on both sides of the partition. Similarly, the stimulating effects of various combinations of sucrose, water, HCl, NaCl and propanol were investigated. The concentrations of HCl and propanol chosen for stimulation were those which, under normal conditions of testing, would yield responses well above the 50% level. The concentration chosen for NaCl was nearer to the 50% level. The number of flies used in each experiment varied from 60 to 800.

In every case, except where noted otherwise, the flies were random sampled, *i.e.*, a different group was tested at each concentration in the series, and the per cent which responded was noted. Median threshold values were calculated according to the method of Bliss (1938) (*cf.* also Dethier and Chadwick, 1948).

RESULTS

Unilateral versus bilateral stimulation by sucrose

Originally a straightforward comparison of the sucrose thresholds of one-legged and two-legged flies was made. In these experiments the flies were tested

in ascending order; that is, each fly was exposed successively to each concentration of the test solution, beginning with a subliminal concentration, until one was reached which elicited a proboscis extension. Since there was a real possibility that one-legged and two-legged flies were not strictly comparable, *i.e.*, that the number of legs immobilized might affect the general behavior of the fly, additional tests were run with two-legged flies, one of whose legs was tethered with a fine silken thread. Each such fly was tested with the tethered leg suspended above the solution (unilateral stimulation) and then with both legs in contact with the solution (bilateral stimulation). Finally the partitioned dish, as already described, was employed. This time the experiments were run in random rather than ascending series. As an added precaution and to insure accuracy of results most of the experiments with partitioned dishes were coded so that the experimenter was unaware of the contents of the dishes.

Regardless of the method of testing employed the results were similar (Table I). The unilateral threshold was always significantly higher than the bilateral threshold (Fig. 1). In these experiments, the absolute values obtained from random testing are not directly comparable with the values obtained from ascending testing because it was not possible to use flies of uniform age and nutritional background throughout the entire study. When these factors are controlled, ascending thresholds are higher than random thresholds (*cf.* Dethier, 1952b).

Contralateral stimulation by unlike compounds

In order first to survey the situation with regard to such representative unacceptable compounds as NaCl, HCl, and propanol, spot tests were undertaken

TABLE I
Comparison of bilateral and unilateral thresholds of response of Phormia regina to sucrose, HCl, and propanol†

Compound and technique of testing‡	Conc. at which 50% of flies respond	Log median ± 2.575 S. E.	$a + S. E.$	$b + S. E.$	\bar{x}	No. of insects tested
Sucrose, one leg, ascending	0.0037	-2.433 \pm 0.191	5.140 \pm 0.050	0.713 \pm 0.082	-2.237	173
Sucrose, two legs, ascending	0.0018	-2.749 \pm 0.149	5.387 \pm 0.055	1.163 \pm 0.122	-2.416	173
Sucrose/water (= 1 leg)	0.1010	-0.996 \pm 0.515	4.326 \pm 0.078	0.640 \pm 0.105	-1.993	360
Sucrose/sucrose (= 2 legs)	0.0200	-1.699 \pm 0.258	4.672 \pm 0.021	0.812 \pm 0.030	-2.103	360
*Sucrose/water (= 1 leg)	0.0177	-1.930 \pm 0.258	4.900 \pm 0.045	0.474 \pm 0.067	-2.141	800
*Sucrose/sucrose (= 2 legs)	0.0021	-2.670 \pm 0.149	5.308 \pm 0.049	0.991 \pm 0.096	-2.359	800
Propanol in water, 1 leg	0.607	-0.217 \pm 0.156	5.037 \pm 0.073	1.206 \pm 0.137	-0.186	360
Propanol in water, 2 legs	1.05	0.020 \pm 0.162	4.734 \pm 0.068	1.160 \pm 0.121	-0.209	420
Propanol/water (= 1 leg)	1.34	0.128 -0.170	5.011 -0.182	2.770 -0.589	0.132	60
Propanol in 0.1 M sucrose						
1 leg	0.59	-0.232 \pm 0.098	5.115 \pm 0.107	2.867 \pm 0.369	-0.192	200
2 legs	0.95	-0.021 \pm 0.098	4.832 \pm 0.105	2.770 \pm 0.351	-0.082	200
Propanol/0.1 M sucrose, random	1.23	0.088 \pm 0.165	5.077 \pm 0.083	1.332 \pm 0.253	0.146	240
Propanol/0.1 M sucrose, ascending	1.076	0.032 \pm 0.155	5.132 \pm 0.134	2.254 \pm 0.311	0.090	120
HCl in 0.1 M sucrose, 1 leg	1.041	-1.391 \pm 0.163	4.877 \pm 0.068	1.107 \pm 0.147	-1.502	70
HCl in 0.1 M sucrose, 2 legs	0.051	-1.298 \pm 0.109	4.773 \pm 0.069	1.441 \pm 0.142	-1.456	70

* As a check against the method all solutions in these series were coded.

† The 4th, 5th, and 6th columns give the calculated values for a , b , and \bar{x} in the equation $Y = a + b(X - \bar{x})$, which is the regression of per cent flies responding, Y , expressed as probits on log concentration, X .

‡ Unless stated otherwise all tests represent random sampling. The slanting line symbolizes a divided dish. For explanation of different sucrose thresholds see text.

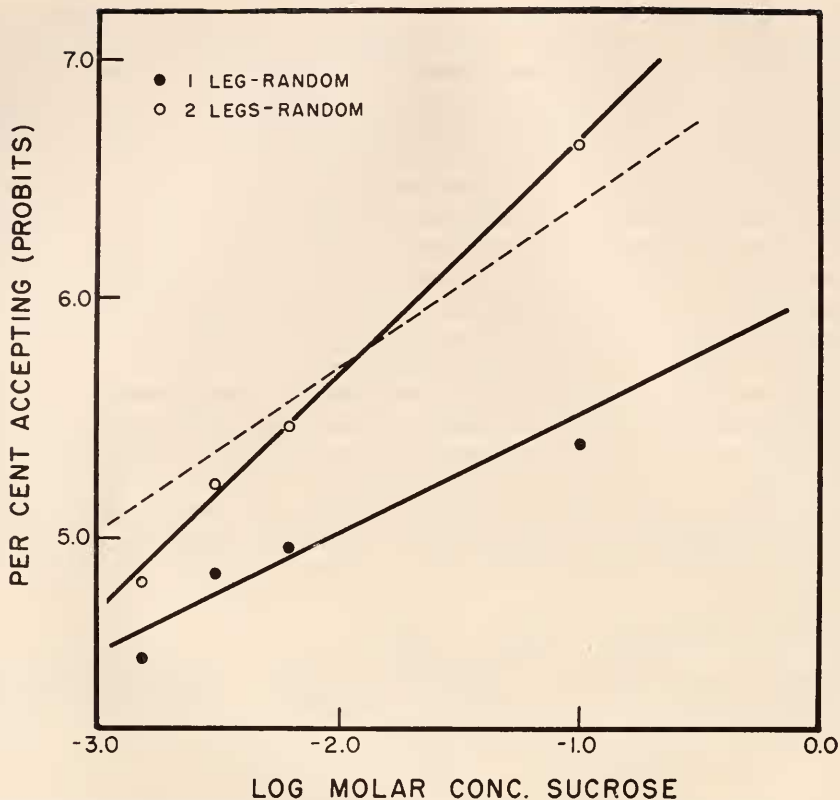


FIGURE 1. Comparison of the distribution of acceptance thresholds for sucrose, as a function of concentration, for flies stimulated unilaterally or bilaterally. The broken line represents the theoretical distribution of bilateral thresholds (two-legged flies) calculated from the expression $1-q^2$ where q equals the fraction of the population of one-legged flies not responding.

by means of a series of partitioned dishes. As had been done earlier in the olfactory studies (Dethier, 1952a) the procedure was adopted of selecting a single concentration at which measurements were made of the change in per cent of the population responding as a different number of appendages was stimulated. This course of action was followed for the sake of simplicity in lieu of varying the concentration to ascertain what strength of stimulus was required to elicit 50% response (median threshold) under each changed experimental condition. However, the quantitative information yielded by such spot testing cannot be utilized to its fullest extent in the absence of additional experiments because there is no way of knowing without further testing what relation the slopes of the threshold distribution curves of one-legged and two-legged flies bear to each other. It will be shown further on that the results of spot tests are substantiated by data from more detailed experiments.

The results obtained with the various spot combinations are arranged in series of pairs (Table II) for purposes of comparison. The following numbers cor-

respond to the comparison numbers in the table, and the nature of the comparison is further indicated here by the symbols in parentheses. A slanting line indicates the partition dividing the two solutions, *s* signifies sucrose, and *P* signifies a repellent compound.

I (H_2O/s vs. P/s). All three unacceptable compounds can prevent a response to sucrose even when acting on the leg opposite to that leg which is being stimulated by sucrose. This is shown by the increase in per cent rejection over that recorded when water alone is paired with sucrose.

TABLE II

Comparison of mean rejection under different conditions of contralateral stimulation

Comparison no.	2 M propanol vs. 0.1 M sucrose and H ₂ O		1 M NaCl vs. 0.1 M sucrose and H ₂ O		0.1 M HCl vs. 0.1 M sucrose and H ₂ O	
	Condition of test	Mean % rejection	Condition of test	Mean % rejection	Condition of test	Mean % rejection
I	H_2O/s	47.2	H_2O/s	58.7*	H_2O/s	46.6
	P/s	70.9	$NaCl/s$	60	HCl/s	65.3
II	$H_2O/P+s$	87.2	$H_2O/NaCl+s$	65.6*	$H_2O/HCl+s$	91.3
	$s/P+s$	54.5	$s/NaCl+s$	53	$s/HCl+s$	73.3
III	$s/P+s$	54.5	$s/NaCl+s$	53*	$s/HCl+s$	73.3
	$P/P+s$	94.5	$NaCl/NaCl+s$	66	$HCl/HCl+s$	99
IV	$H_2O/P+s$	87.2	$H_2O/NaCl+s$	65.6*	$H_2O/HCl+s$	91.3
	P/s	70.9	$NaCl/s$	60	HCl/s	65.3
V	P/s	70.9	$NaCl/s$	60*	HCl/s	65.3
	$P/s+P$	94.5	$NaCl/s+NaCl$	66	$HCl/s+HCl$	99
VI	$P+s/P$	94.5†	$NaCl+s/NaCl$	66*	$HCl+s/HCl$	99†
	$P+s/H_2O$	87.2	$NaCl+s/H_2O$	65.6	$HCl+s/H_2O$	91.3
n	110		160		150	

* There is no significant difference between the means compared in these pairs.

† Difference is significant at $P = 0.02$.

‡ Difference is significant at $P = 0.05$. All other differences are highly significant.

n = number of flies tested in each paired comparison.

II ($H_2O/P+s$ vs. $S/P+s$). An appreciably lower rejection is obtained with sucrose on both sides of the partition and a repellent compound on one side only. This result is in agreement with the fact that bilateral stimulation by sucrose is followed by greater acceptance than is unilateral stimulation.

III ($s/P+s$ vs. $P/P+s$). This comparison seems to show that there is a greater rejection when two legs are stimulated by unacceptable compounds than when a single leg is stimulated. This apparent summation is attributable, at least in part, to the difference with respect to sucrose stimulation. Note that in one member of the pair there is sucrose on both sides of the partition while in the other it is on one side only. This result agrees with II above.

IV ($H_2O/P + s$ vs. P/s). Greater rejection is obtained when opposing compounds stimulate the same leg than when they act independently on opposite legs. This effect may be due either to some inhibiting action of the repellent compound on the sugar receptors over and above its independent effect (see I) or to some "loss" of repellent effectiveness as a result of looser central integration when opposing stimuli act on different sides.

V (P/s vs. $P/s + P$). There is greater rejection when the unacceptable compound acts on both legs instead of on one. Part of the observed difference may result from the fact that in one dish P and s are acting independently as well as contralaterally and that ipsilateral repellency is greater than contralateral repellency as shown in IV above.

VI ($P + s/P$ vs. $P + s/H_2O$). When the sugar is identical in both tests (here sucrose is on one side only), the repellency is slightly but consistently higher with the repellent on both sides of the partition. This experiment shows that for unacceptable compounds the bilateral threshold is lower than the unilateral threshold.

Unilateral versus bilateral stimulation by unacceptable compounds

As has already been pointed out, a critical comparison of unilateral versus bilateral thresholds must be based upon complete threshold frequency distribution curves and not merely upon responses to a single selected concentration. Consequently, as a check on the foregoing results, especially comparison VI, and with propanol and HCl as models, tests were run over complete concentration ranges. With propanol, tests were run with one-legged flies, two-legged flies, and with partitioned dishes. Propanol was tested with and without the addition of sucrose. With HCl, tests were run with one-legged and two-legged flies. In each case 0.1 *M* sucrose was mixed with the HCl. Data are summarized in Table I. Three facts emerge clearly from an examination of these data: (1) there is no statistically significant difference between unilateral and bilateral thresholds for either propanol or HCl; (2) nevertheless, unilateral thresholds have a slight but consistent tendency to be lower than bilateral thresholds; (3) there is no significant difference of threshold for propanol in water and in sucrose.

DISCUSSION

As early as 1938 the experiments (Table 16) of Inamura in which tarsal receptors of the fly *Sturmia sericariae* Cornalia were stimulated by sugar had given an intimation of an increased sensitivity to bilateral stimulation over unilateral. Studies of olfactory thresholds of *Phormia regina* (Dethier, 1952a) had yielded data which suggested the possibility of a precise quantitative relationship between bilateral and unilateral thresholds. It is now clear that such quantitative relationships exist. But although it can be proven that for any individual the bilateral thresholds for certain compounds are significantly lower than the unilateral thresholds, it is by no means certain that the increased sensitivity is attributable to contralateral summation in the usually accepted sense.

In the analogous case, where comparisons have been made between monocular and binocular vision, Pirenne (1943) and Bárány (1946a, 1946b) have pointed

out that the experimental procedure by its very nature assures that the two eyes will see more clearly than one. "Let us assume that the visual acuities (or other thresholds) of both eyes fluctuate independently of one another . . . and that the instantaneous thresholds for monocular vision have the same distribution in both eyes, . . . then if the one eye alone has the chance a of seeing the symbol, both eyes together have the chance $2a - a^2$. As a is smaller than 1, this expression will always be greater than a —that is to say, two eyes will be able to see better than one solely as a result of random combination" (Bárány, 1946b, p. 127). And, as Smith and Licklider (1949) go on to state, the same source of bias is inherent in the procedure as applied to the determination of thresholds in other sense modalities. The idea can be clarified still further by quoting from the study of hearing by these authors (p. 279). "In order to estimate the magnitude of the bias, it is necessary to define the null condition under which we should say that there is no binaural summation. We can imagine, for this purpose, two monaural listeners, one with only a right ear, the other with only a left ear. The two listeners have no means whatsoever of communicating with each other, but both report to the same experimenter. To obtain measures of monaural and 'binaural' sensitivity, the experimenter tests the two listeners separately (successively), then together, in the latter instance recording a positive response whenever *either* listener reports hearing the stimulus tone. It need hardly be said that we do not propose this schema as a psychophysiological hypothesis. There is of course no doubt that the two ears of a single listener do send their messages to a common center in which true summation may occur. Evidence of interaural interaction has been found in psychophysical experiments on sound localization and on masking and in electrophysiological observations at the levels of the cochlear nucleus and the temporal lobe. The schema is therefore a 'null hypothesis' [of supplementation] which a given set of data should lead us to reject before we say that the data constitute evidence of true binaural summation."

The applicability of this hypothesis of supplementation to the present case is easily tested. Let us assume that x number of sucrose receptors on the tarsi must be activated in order to insure a response, that is, that there is intra-leg summation. Then at the median acceptance threshold value 50% of the flies have x or more receptors acting. The probability at this concentration that a fly (x or more active receptors) will be in the half of the population which is responding is 50%. This probability can be raised either by increasing the concentration or by increasing the number of available receptors (n). If n is doubled, the probability of a response occurring increases. The increase can be calculated from the expression $1 - q^2$ where q is equal to the fraction not acting at the median concentration. Accordingly, the concentration which elicits a response from 50% of the one-legged flies should elicit a response from 75% of the two-legged flies if there is no interaction between the two legs. In the present experiments this expectation is realized. As a visual examination of Figure 1 shows, the response of the two-legged flies is not greater, except at the highest concentrations, than that which would be predicted on a probability basis from the behavior of one-legged individuals. The difference between the expected line (calculated from the line for one-legged thresholds from the expression $1 - q^2$ where q = the fraction not responding) and the line describing actual two-legged thresholds is not significant. Thus the data do not constitute evidence of true contralateral summation. Never-

theless, as was pointed out in the case of olfactory responses (Dethier, 1952a), the results represent from the point of view of the integrated organism a behavioral summation of no little importance.

The action of unacceptable compounds (propanol, HCl, NaCl) on one-legged and two-legged flies appears at first to follow an entirely different pattern from that observed with sucrose. Bilateral thresholds are not lower than unilateral

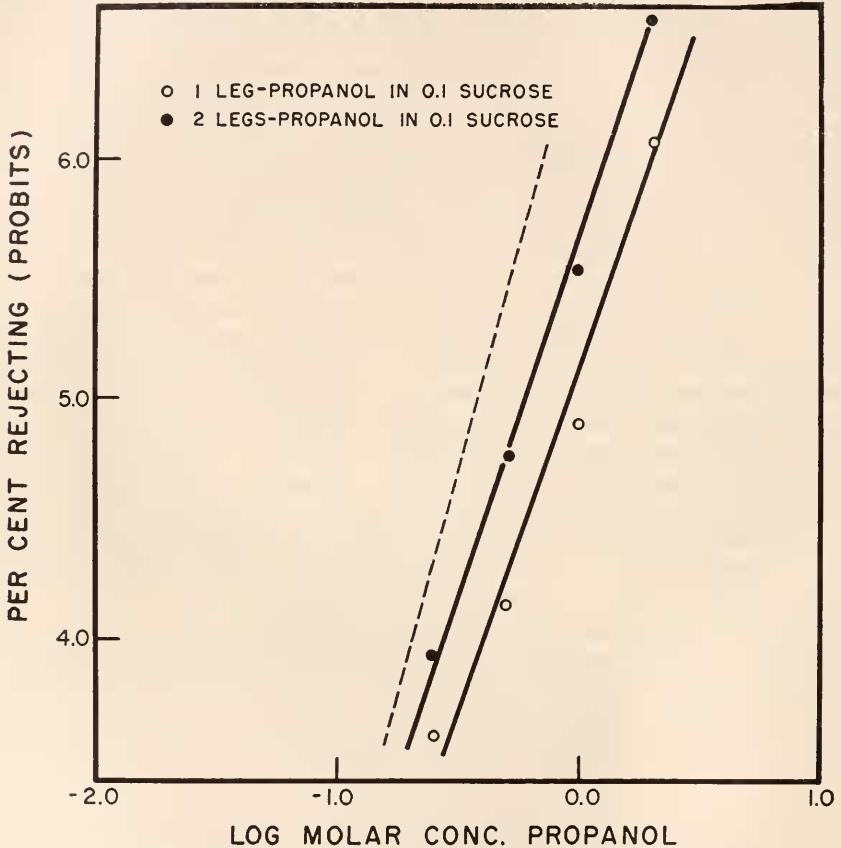


FIGURE 2. Comparison of the distribution of rejection thresholds for propanol, as a function of concentration, for flies stimulated unilaterally or bilaterally. The broken line represents the theoretical distribution of bilateral thresholds (two-legged flies) calculated from the expression $1-q^2$ where q equals the fraction of the population of one-legged flies not responding.

thresholds as might have been expected and as the spot tests (comparison VI) show. Actually, data from the spot tests and from complete concentration tests are in agreement, and supplementation occurs in much the same manner as it does with sucrose. The following explanation of the one-legged and two-legged fly experiments should clarify this point: (1) less sucrose is required to elicit a response from two-legged than from one-legged flies; (2) in most of the experiments with propanol and HCl, the test solutions contained 0.1 M sucrose regardless

of the number of legs to be stimulated; therefore, since the sucrose "sums" contralaterally, there was actually a greater effect for the propanol and HCl to overcome in two-legged flies than in one-legged flies; (3) if propanol and HCl "sum" as sucrose does, the greater sucrose effect would be overcome with the same concentrations of propanol or HCl in the one-legged and the two-legged flies; in other words, the rejection thresholds should be the same regardless of the number of legs stimulated; (4) if neither propanol nor HCl "sums," then a greater concentration of these compounds would be required on a two-legged fly to overcome the increased sucrose effect, and, therefore, propanol and HCl thresholds should be higher with two-legged than with one-legged flies.

A comparison of thresholds of one-legged and two-legged flies for unacceptable compounds reveals no statistically significant difference between the two although a slight but consistent tendency for the bilateral threshold to be higher has been commented upon. It would be expected, however, that if this tendency were truly an expression of a failure to sum or supplement, as outlined under (4) in the preceding paragraph, the difference would be of the same order of magnitude as that found with unilateral and bilateral sucrose thresholds but of the opposite sign. Obviously the observed difference is too small. The alternative suggestion, that unacceptable compounds do indeed "sum" in a manner similar to sucrose, though possibly not to the same extent, fits the data with fewer contradictions. This interpretation is strongly supported by the spot tests in comparison VI ($P + s/P$ vs. $P + s/H^2O$) where the sugar concentrations are controlled in such a way that the difference in unilateral and bilateral thresholds shows up directly.

Furthermore, this interpretation agrees with the results obtained in olfactory tests where the unacceptable compound pentanol was found to stimulate a fly with two antennae more effectively than it did a fly with one antenna (Dethier, 1952a). The reason that a threshold difference could be demonstrated directly in the olfactory tests is explained by the fact that the pentanol vapor was not counteracting the effect of an acceptable compound. The pentanol rejection threshold represented the concentration necessary to drive the flies away from light. When one antenna was amputated in these experiments, the number of receptors being stimulated by light was in no way affected. On the other hand, immobilization of one leg in the tarsal experiments not only reduced the number of receptors being stimulated by the unacceptable compound but also the number affected by the acceptable compound.

The foregoing considerations involving tarsi have been based principally upon those cases in which the unacceptable compounds were mixed with sucrose. An examination of Table I will show that comparable results were obtained with propanol when sucrose was absent from the test solutions. Determinations of the rejection thresholds of thirsty flies for propanol in water gave unilateral and bilateral threshold values which were not significantly different from each other. By applying the same reasoning to data obtained from water mixtures as was applied above to data relating to sucrose mixtures, one arrives at the conclusion that just as there is supplementation of response to sucrose, so also is there supplementation with water. Since the concentration of water cannot be altered, direct demonstration of the phenomenon of supplementation is impossible in this case.

In the sense that propanol, HCl, and NaCl alter the normal pattern of activity from tarsal receptors, they may be said to be stimulating. While a conclusion of this sort is hardly unexpected insofar as the electrolytes are concerned, some find it difficult to envision alcohol as a stimulus to sensory structures. Yet observations of the reactions of individual flies show that in solutions containing alcohol there is agitated movement of the feet, even attempts at withdrawal, and that all gradations exist between absolute failure to extend the proboscis, complete extension followed immediately by exceptionally rapid withdrawal, and partial rather hesitant extension. In other studies, many species of insects have been shown to be stimulated by alcohols. The ovipositors (which are insensitive to water) of certain hymenopterous parasites and of the cricket (*Gryllus*) are excited by inorganic electrolytes and aliphatic alcohols. Application of threshold concentrations results in characteristic movements of the organ (Dethier, 1947, 1951). The beetle *Laccophilus* is stimulated to greater swimming activity by alcohols (Hodgson, 1951). *Phormia* is stimulated via the antennae to greater flying activity by alcohol vapors (Dethier and Yost, 1952), and *Drosophila* (Reed, 1938) is attracted by low concentrations of short-chain aliphatic alcohols. On the other hand, it is also known that short exposure to high concentrations or prolonged exposure to lower concentrations of alcohols will result in narcosis. What may be narcosis of tarsal sugar receptors has been observed in *Phormia* (cf. Dethier, 1951).

From the neurophysiological point of view the action of a compound like alcohol could be either excitatory or depressant, or excitatory at one concentration and depressant at another. Peripheral inhibition of activity in sense organs by a stimulus as a means of "stimulating" an animal has been recorded sporadically. Granit (1947) and others have described fibers of the retina in which there is commonly spontaneous activity which may be depressed by illumination. Löwenstein and Sand (1940) have reported that spontaneous discharges from single fibers from the horizontal ampulla of the isolated labyrinth of *Raja* are increased by ipsilateral stimulation and inhibited by contralateral stimulation.

Ultimately the revelation of the nature of those events which are occurring peripherally in *Phormia* at the time of stimulation can be realized probably only through the agency of electrophysiological techniques. However, the experiments reported here foreshadow to some extent the nature of these events. Data from experiments with divided dishes do not in themselves prove that unacceptable compounds and sucrose act upon different receptors, but taken in conjunction with other experiments they lend strong support to the idea that there are at least two types of tarsal chemoreceptors. Admittedly, it could be argued that only a single type of receptors need be postulated to explain the facts. An argument along these lines, which could apply equally well to ipsilateral and contralateral stimulation by opposing stimuli, would require that sucrose stimulate the receptors and that alcohol depress them. It would require further that there be spontaneous activity from the receptors and that any decrease in the basal level of discharge from one leg could offset in the central nervous system the increased activity from the opposite leg which was undergoing stimulation by sucrose. According to this scheme, however, any factor which could depress spontaneous activity in one leg should be able to prevent proboscis response when the opposite leg is stimulated by sucrose. Amputating a leg would be a most effective means of obliterating spontaneous activity. Yet, when

this is done, stimulation of the remaining prothoracic leg by a threshold amount (for unilateral stimulation) of sucrose elicits the usual proboscis response.

Another experimental result which is not in accord with the one-receptor-type hypothesis is the quantitative difference between supplementation with sucrose and supplementation with unacceptable compounds. With sucrose the difference between unilateral and bilateral thresholds is considerable; with unacceptable compounds it is difficult to demonstrate, even in the absence of such disturbing influences as water and sucrose (*cf.* spot test comparison VI). It would be expected that supplementation would be of equal value with acceptable and unacceptable compounds if these were acting on the same population of receptors.

In view of the objections just stated, it is difficult to reconcile the available data with a one-type-receptor hypothesis. All of the results reported are more consistent with the idea that sucrose and unacceptable compounds act on different populations of receptors. It is to be hoped that the tarsal receptors may eventually yield to attack by electrophysiological methods.

SUMMARY

Acceptance thresholds of the tarsal chemoreceptors of the blowfly, *Phormia regina*, for sucrose and rejection thresholds for HCl, NaCl, and propanol have been determined. Comparisons were made of the thresholds of one-legged and two-legged flies. Comparisons were also made of rejection thresholds determined (a) on one leg exposed to a mixture of sucrose and unacceptable compound and (b) on two contralateral legs, one of which was exposed to sucrose alone and the other to an unacceptable compound alone, the exposure being simultaneous. The following results were obtained:

- (1) The bilateral threshold for sucrose is lower than the unilateral threshold.
- (2) For sucrose the decrease of bilateral over unilateral threshold is never greater than can be satisfactorily accounted for on a simple probability basis.
- (3) Unacceptable compounds terminate the response to sucrose even when applied to the leg not receiving sucrose, *i.e.*, they can act contralaterally as well as ipsilaterally.
- (4) There is a greater rejection when two opposing stimuli act on one leg than when they stimulate two contralateral legs.
- (5) Unacceptable compounds also "sum" contralaterally.
- (6) Supplementation also occurs in the response of thirsty flies to water.
- (7) The prevention of proboscis extension to water and sucrose by unacceptable compounds is predominantly a central phenomenon.
- (8) Available evidence favors the interpretation that there are at least two distinct sets of receptors on the tarsi, sugar receptors and non-sugar receptors.

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